

## TRANSFORMATION OF ORGANIC SUBSTANCES IN SOIL

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16. Abstract The article presents the results of several series of tests on mineralization and humification of organic matter in the soil, as functions of several variables, including adding materials of varying complexity (starch, casein, alfalfa meal, wheat straw, cellulose), aeration conditions and use of N and P alone and together, as well as with and without manure ap- plication to the soil. A considerable amount of test data is presented in tables and graphs, and several numerical ratios of various test parameters. Results are explained in terms of various simple thermodynamic relationships, especially as to high-energy phosphate bonds and ATP and their involvement in biological activity. The results are said to be useful in preparation of organic fertilizers and regulation of change in organic substances in the soil by microorganisms.			
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## TRANSFORMATION OF ORGANIC SUBSTANCES IN SOIL

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In regions of the Czechoslovakian SSR with intensive agri- /106\*  
culture, 40-50 centner/hectare of dry organic matter enters the soil  
annually, approximately half of it in the form of organic ferti-  
lizers [Novák, 26].

Although the soil receives a comparatively large amount of  
organic matter, the amount of soil humus remains almost the same and,  
sometimes, it even decreases a little [Novák, 27; Stranak, 56].

Some authors [Scheffer, Ulrich, 54; Liesche, 18 et al.] think  
that the amount of soil humus does not depend on the amount of  
organic matter introduced into the soil. The results of short-term  
and long-term tests with fertilizers do not confirm this. As a rule,  
plots with organic fertilizer contain more humus than plots, which  
are completely unfertilized. The increase in amount of humus in the  
soil will usually be proportional to the amount of organic ferti-  
lizer applied [Apfelthaler, Novák, 1; Michael, Djubari, 19; Nehring,  
Wiesemüller, 25; Rauhe, 47, 48, 19].

Other authors state that, although the amount of humus in the  
soil does not depend on the amount of organic matter in the ferti-  
lizer, it does depend on the quality of it [Sauerbeck, 50, 51;  
Oberländer, Roth, 42; Zelleretal, 57 et al.]. The results of our  
test show that this opinion is incorrect. The amount of organic  
matter in the organic fertilizers used strongly affects the intensity  
and productivity of humification [Novák, 28-36; Novák et al., 40;  
et al.].

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\*Numbers in the margin indicate pagination in the foreign text.

An entire series of other factors (degree of aeration, concentration and mutual relationship of the biogenic elements, the amount and species composition of the microbes, soil reactions, etc.), of course, affect mineralization and humification of organic matter in the soil. Some of the relations studied in our Institute are presented in this work.

We present the results of a laboratory test of addition to /107 the soil of starch, casein and ground alfalfa, in the amount of 5% C by weight of dry soil, in Fig. 1. The C:N ratio was 3, and it was equalized in all versions of the test, by addition of ammonium carbonate. The test lasted for six weeks, at a temperature of 28°.

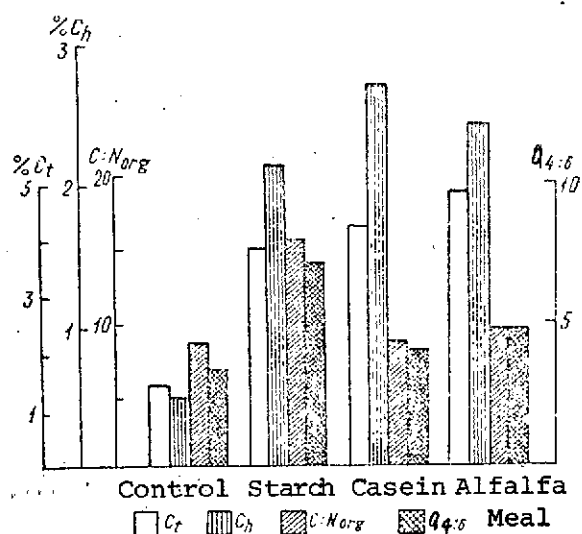


Fig. 1. Total C ( $C_t$ ), C of the total humic acid and fulvic acid ( $C_h$ ) content, C:N organic ratio and humic acid extraction coefficient ( $Q_{4:6}$ )

the test with ground alfalfa added then follows, 2.39% C. In the starch test, only 2.14% humic acid carbon was found and, in the control test, 0.537% humic acid C.

The ratio of organic carbon to organic nitrogen is represented in the next column. In humification, the change of N into organic bonds takes place up to approximately a C:N ratio of 10. If the

In the test with addition of starch, 2.51% of the carbon, i.e., over 50%, was mineralized. In the test with casein added, 2.16% of the carbon, i.e., approximately 43%, was mineralized, and in the test with ground alfalfa added, only 1.74% of the carbon, i.e., approximately 25%, was mineralized.

The sum of the humic acids and fulvic acids at the end of the test was the greatest in the test with casein added, 2.70% C, and

C:N<sub>org</sub> ratio is greater than 10, this means that the humification process still is not completed and that organic matter not containing nitrogen is relatively predominant in the system. This is /108 expressed particularly clearly in the starch addition test.

In the last columns of this figure, the values of the light coefficient  $Q_{4:6}$  of extracted humic acids are introduced. It turns out that the least condensed molecules of humic acid are found in the starch addition test, that the most condensed are found in the control, and that the casein addition test follows it.

The amount of humic acid and fulvic acid extracted increases in all cases, with addition of organic substrates. The most intense humic acid synthesis took place in the casein addition test and the least, in the starch test, but the greatest amount of fulvic acid formed in this test. The stability of the organic matter in the control test was the greatest. The relative stability of organic matter in the soil decreases with addition of organic substrates. Organic matter turned out to be the least stabilized in the starch addition test.

Casein displayed the optimum effect of all the substrates on humification rate (production of humus materials per unit time). The productivity of humification, production of humus matter formed per unit of mineralized substrate, is important.

By utilization and mutual comparison of substrates, for which a sufficient amount of thermodynamic data are known, especially if the Gibbs free energy of mineralization is known, we express the productivity of humification by the amount of humic acid synthesized per unit of free energy freed by mineralization of the substrate. If the humification productivity is calculated on the basis of these principles, it turns out that the humification productivity with casein is 56% greater than with ground alfalfa and 75% greater than with starch. These results are in complete agreement with our hypothesis of the formation of humus [Novák, 29-31], based on the ratio of the endothermic and exothermic reactions in the synthesis of

humus matter from the substrates. It is evident that the scale of the synthesis necessary will be less, just like the total consumption of energy introduced into the system from outside by consumption of substrate, which, if even partially similar to the soil humus, or, where monomers, which are similar to or identical to the humus monomers can be found, than in the case when the substrate is completely unlike humus. This was confirmed in our test. Starch had the least humification productivity and ground alfalfa, the greatest. Similar results have been obtained in other tests [Novák, 109 32-34a, b and c; 35, 36a and b, 37-39; Novák, Nováková, 47; Apfelthaler, Novák, 7].

In tests with different aeration and various degrees of aeration, several substrates were tried. The clearest results usually are obtained, by use of substrates dissolved or suspended in a nutrient solution or which are in sand cultivation. Inoculation with soil infusions proved to be advantageous in these tests.

We present the results of tests using straw and cellulose as substrate.

### Test Procedures

The soil infusion was prepared by shaking 1 part of earth with 100 parts of sterile tap water for a period of one hour. After /110 letting the suspension settle for a period of one hour, the supernatant liquid was used in the tests. 0.6%  $(\text{NH}_4)_2\text{SO}_4$  was dissolved in this infusion and 10% sterile, finely ground wheat straw or 10% of finely ground cellulose was suspended in it.

The test lasted 12 weeks at 28°. Half the containers were maintained under aerobic conditions (air was added continuously, in the amount of about 2 l per 1 l of suspension per hour) and the second half, under anaerobic conditions (it was treated with  $\text{N}_2$  and sealed with fermentation tongues).

The respiration values (Table 1), especially the relative respiration rates, show that the clearly visible formation of humus, indicating an increasing stability of the organic matter, takes

TABLE 1  
Respiration values of test versions with  
aerobic and anaerobic incubation of straw and  
cellulose under laboratory conditions

	Straw		Cellulose	
	Aerobic	Anaerobic	Aerobic	Anaerobic
mg CO <sub>2</sub> /100 g C per hour				
B—control	3.0	4.0	3.0	3.6
B+N (20 mg N in ammonium form)	6.4	1.8	3.3	3.9
B+G (glucose + 200 mg C)	7.2	2.8	6.2	4.5
B+Pep (peptone 200 mg C)	37.6	21.1	72.0	21.5
B+P (equivalent mix. KH <sub>2</sub> PO <sub>4</sub> and Na <sub>2</sub> HPO <sub>4</sub> )	1.6	4.9	4.5	3.1
B+NG	7.8	1.9	5.5	4.8
B+NP	2.5	4.7	3.4	5.1
B+GP	7.6	5.3	7.3	4.6
B+NGP	8.6	5.1	6.1	4.8
B+PepP	68.3	18.2	80.4	20.5
Ratios of characteristics of various test versions				
N : B	0.8	0.45	1.1	1.08
G : B	2.4	0.7	2.07	1.25
NG : B	2.6	0.48	1.83	1.33
Pep : B	12.5	5.27	24.0	5.98
P : B	0.53	1.23	1.5	0.86
NP : B	0.83	1.18	1.13	1.42
GP : B	2.54	1.32	2.43	1.28
NGP : B	2.86	1.27	2.03	1.33
PepP : B	22.7	4.56	26.7	5.69
NP : P	1.56	0.96	0.76	1.64
GP : P	4.75	1.08	1.62	1.48
NGP : P	5.37	1.04	1.36	1.55
PepP : P	42.6	3.72	17.8	6.62

place only in the aerobic versions. By comparing the corresponding versions of aerobic and anaerobic substrates, we can draw the following conclusions.

The relative respiration rates, especially in those cases when an organic substrate was provided, will always be higher in the versions maintained under aerobic conditions. The ratio of the C:B values of straw, in the aerobic and anaerobic versions:

$$\frac{G(SO_2) : B(SO_2)}{G(SN_2) : B(SN_2)} = 3.44$$

where the index  $SO_2$  is straw maintained under aerobic conditions and  $SN_2$  is straw maintained under anaerobic conditions. We find the following ratio in the quantity NG:B:

$$\frac{NG(SO_2) : B(SO_2)}{NG(SN_2) : B(SN_2)} = 5.42$$

The following ratios can be derived for the remaining important quantities:

$$\begin{array}{l} \frac{\text{Pep}(\text{SO}_2) : \text{B}(\text{SO}_2)}{\text{Pep}(\text{SN}_2) : \text{B}(\text{SN}_2)} = 2.38 \\ \frac{\text{GP}(\text{SO}_2) : \text{B}(\text{SO}_2)}{\text{GP}(\text{SN}_2) : \text{B}(\text{SN}_2)} = 1.93 \\ \frac{\text{NGP}(\text{SO}_2) : \text{B}(\text{SO}_2)}{\text{NGP}(\text{SN}_2) : \text{B}(\text{SN}_2)} = 2.25 \\ \frac{\text{PepP}(\text{SO}_2) : \text{B}(\text{SO}_2)}{\text{PepP}(\text{SN}_2) : \text{B}(\text{SN}_2)} = 4.98 \end{array}$$

For the corresponding values in the versions with cellulose, we obtain the following expressions:

$$\frac{\text{G}(\text{CO}_2) : \text{B}(\text{CO}_2)}{\text{G}(\text{CN}_2) : \text{B}(\text{CN}_2)} = 4.30$$

where the index  $\text{CO}_2$  is cellulose maintained under aerobic conditions and  $\text{CN}_2$  is cellulose maintained under anaerobic conditions: /111

$$\begin{array}{l} \frac{\text{NG}(\text{CO}_2) : \text{B}(\text{CO}_2)}{\text{NG}(\text{CN}_2) : \text{B}(\text{CN}_2)} = 3.89 \\ \frac{\text{Pep}(\text{CO}_2) : \text{B}(\text{CO}_2)}{\text{Pep}(\text{CN}_2) : \text{B}(\text{CN}_2)} = 4.03 \\ \frac{\text{GP}(\text{CO}_2) : \text{B}(\text{CO}_2)}{\text{GP}(\text{CN}_2) : \text{B}(\text{CN}_2)} = 4.52 \\ \frac{\text{NGP}(\text{CO}_2) : \text{B}(\text{CO}_2)}{\text{NGP}(\text{CN}_2) : \text{B}(\text{CN}_2)} = 4.04 \\ \frac{\text{PepP}(\text{CO}_2) : \text{B}(\text{CO}_2)}{\text{PepP}(\text{CN}_2) : \text{B}(\text{CN}_2)} = 5.26 \end{array}$$

All the biochemical indicators of humus stability indicate more favorable humification under aerobic conditions.

A great effect of the substrate was not found on the relative increase in humus stability under aerobic conditions, compared with anaerobic conditions. In this case, the total humification yield in versions with straw was considerably greater than in versions with cellulose (Table 2). This corresponds to the results of our previous tests [Novák, 39].

TABLE 2  
Analytical values of test versions with aerobic  
and anaerobic incubation of straw and cellulose

Characteristic	Straw		Cellulose	
	Aerobic	Anaerobic	Aerobic	Anaerobic
1. Dry wt. (%) . . .	6.18	8.26	5.81	6.96
2. C (%) . . .	3.04	3.63	2.89	2.97
3. C-ha. (mg C/100 ml)				
1071 . . .	1071	225	134	0
4. C-ha. . .	399	327	192	86
5. C-ha.+C-fa. . .	1470	552	326	86
6. C-Ha.:C-fa. . .	2.69	0.69	0.70	—
7. Q <sub>4</sub> :7 . . .	5.3	11.4	7.9	—
8. C-ha. B % C . . .	35.3	6.2	4.64	—
9. C-fa. B % C . . .	13.1	9.02	6.66	2.90
10. C-ha.+C-fa. . .	48.4	15.22	11.30	2.90
B % C . . .				
11. C in % of dry weight . . .	49.4	44.0	49.8	42.8

It can be concluded from the data of analysis of the test /112  
that losses of organic matter during the test were the following in  
the individual versions: straw, aerobic incubation, 38.2%; straw,  
anaerobic incubation, 17.4%; cellulose, aerobic incubation, 41.9%;  
cellulose, anaerobic incubation, 30.4%.

If it is assumed that the average carbon content in humic acids  
is approximately 55% [Kononova, 12, 13; Flaig, 3; Scheffer, Ulrich,  
54; Naimr, 24, et al.], the amount of humic acid extracted in indi-  
vidual versions will be the following: straw, aerobic incubation,  
26.8%; straw, anaerobic incubation, 100%; cellulose, aerobic incu-  
bation, 5.95%; cellulose, anaerobic incubation, 1.56%.

Considerable amounts of humic acids were obtained in the straw  
versions. 1.87% of the humic acid C and 2.86% of the fulvic acid C  
could be extracted from fresh straw, or 8.63% humic acid (with 55%  
C). This amount must be subtracted in both versions with straw as  
the substrate, in order to obtain the "pure" production of humic  
acids. This is 18.17% in the aerobic version and 1.37% in the  
anaerobic.

The humification productivity can be calculated from these values, in a manner, similar to the way it was done in the preceding tests [Novák, 39]:

$$\begin{aligned} P(\text{CO}_2) - \text{h.a.} + \text{f.a.} &= 48.7\% \\ P(\text{SN}_2) - \text{h.a.} + \text{f.a.} &= 7.85\% \\ P(\text{CO}_2) - \text{h.a.} + \text{f.a.} &= 14.3\% \\ P(\text{CN}_2) - \text{h.a.} + \text{f.a.} &= 5.15\% \end{aligned}$$

For a comparison of the corresponding aerobic and anaerobic versions, we obtain the ratio for straw as the substrate:

$$P(\text{SO}_2) : P(\text{SN}_2) = 6.1 : 1$$

For cellulose as the substrate we obtain the ratio:

$$P(\text{CO}_2) : P(\text{CN}_2) = 2.78 : 1$$

Thus, aerobic humification proves to be six times more productive than anaerobic in the straw versions and three times, in the cellulose versions. It must still be added that the ratios of humic acids and fulvic acids in both aerobic versions turn out to be more favorable than in the anaerobic versions (Table 2); humic acid was not formed at all in the anaerobic cellulose version. The degree of condensation of humic acids, determined on the basis of optical properties, was greater in the aerobic versions. An estimate can be made from the same test of the effect of the substrate on humification. From the point of view of humification productivity, we obtain the following ratio in the aerobic versions:

$$P(\text{SO}_2) : P(\text{CO}_2) = 3.41 : 1$$

and in the anaerobic versions:

$$P(\text{SN}_2) : P(\text{CN}_2) = 1.52 : 1$$

In this test, the effect of substrate quality of humification /113 is confirmed [Novák, 39]. Straw is a more complex substrate, and a considerable portion of the humus matter monomers can be freed by simple hydrolytic processes, while only glucose is the cellulose monomer, which must be transformed considerably in humification.

A very important circumstance is that the relative effect of substrate is expressed very much more under aerobic conditions than under anaerobic. This is in complete agreement with the theory expressed, because, under anaerobic conditions, under the effect of insufficient liberation of energy, the total humus production will be small; therefore, the difference in humification productivity between the various substrates cannot be great.

Rather, under anaerobic conditions, the effect of the substrate appears in the interrelation between the humic acids and fulvic acids formed, which turns out to be more favorable in straw (complex substrate) than in cellulose (simple substrate) in our test.

The results obtained with model substrates were confirmed in a test with aerobic and anaerobic manure-spreading. Since addition of earth to manure had a much better effect in all our preceding tests, we included versions in the test, without addition of earth to the manure and with addition of 10% earth by weight of manure. The test was concluded after four weeks of aging.

The decrease in organic matter was determined by two methods: by analysis of the initial material and the final products for ash content (the absolute amount of ash does not change) and carbon in the dry matter, as well as by the decrease in carbon, in the form of  $\text{CO}_2$ , during the entire test. The results of both analyses are very close together.

The losses of organic matter under aerobic conditions is 3-4 times greater than under anaerobic. Addition of earth decreases mineralization of the substrate somewhat under aerobic conditions.

TABLE 3  
Decrease in organic carbon

Test version	Calculated	
	by $CO_2$ production	by analysis of raw materials and products
	%	
Manure, aerobic conditions	16.8	14.7
anaerobic	4.1	5.9
+ earth, aerobic conditions	14.6	12.2
+ anaerobic	4.5	5.2

A more pronounced difference was found between the individual versions in production of humus matter. The data of this test are introduced in Figure 2. The decrease in carbon is expressed in the first columns of each version. The second columns represent pure production of humic acids (the concept of "pure production" of humic acids should be considered to be the amount of humic acids and fulvic acids during the test, expressed in weight units of the initial raw material used).

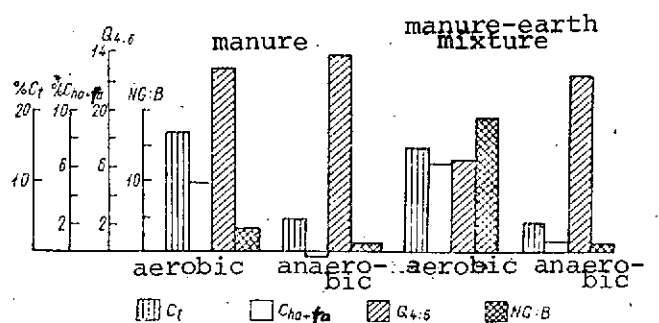


Fig. 2. Characteristics of manure, applied under aerobic and anaerobic conditions, and mixtures of manure with earth (10:1):  $C_t$  = decrease in C;  $C_{ha-fa}$  = C forming humic acids;  $Q$  is the light coefficient of the humic acids;  $N:B$  is the coefficient of potential and basal respiration increased only negligibly. Under anaerobic conditions, humic acid production was significant, especially in the version with earth added.

In the course of the test, the amount of humic acids changed little under anaerobic conditions. If the total decrease in organic matter is kept in mind, it turns out that, in the anaerobic version, without addition of earth, the absolute amount of humic acids decreased during the aging. In manure with earth added under anaerobic conditions, this amount

Although under anaerobic conditions, the total decrease in organic matter is considerably less than under aerobic, the humification productivity is greater under aerobic conditions.

The values of the light coefficients of the extracted humic acids show that, only with application of a mixture of manure and earth under aerobic conditions, do humic acids similar to the soil humic acids form.

In this manner, anaerobic processes do not cause production of humus, or this production will be negligible. In aerobic processes, 115 humus always is formed. A number of authors, however, are of the opinion that aerobic processes are not the optimum ones for humification under all circumstances [Stoklasa, 55; Novák, 29-31a, b; Cizek, 2; et al.].

On the basis of our working hypothesis of humus formation, it can be assumed that total aeration, especially of complex organic substrates, in which humification does not require such a great synthesis scale, as well as exogenous sources of free energy, as humification of simple materials, leads to a lower rate and, perhaps, humification productivity, than partially limited aeration.

In developing test procedures, which could confirm our opinion, we proceeded from the fact that low molecular weight organic matter, mainly intermediate metabolites of the anaerobic process of change of matter, can be relatively easily preserved in the experimental system. Retention of energy in a biochemically usable form can be accomplished only on a limited scale. Our opinion was confirmed in preliminary tests.

Therefore, we conducted tests, with prior anaerobic incubation of a mixture of manure and earth, with subsequent incubation under aerobic conditions. The preliminary incubation lasted 10-20 days, and the entire test lasted 60 days. Aerobic and anaerobic versions were introduced as controls.

- Version A - 60 days aerobic incubation
- " B - 10 days anaerobic and 50 days aerobic incubation
- " C - 20 days anaerobic and 40 days aerobic incubation
- " D - 60 days anaerobic incubation.

The amount of mineralized organic matter in the initial substrate is designated by the first columns in each version in Fig. 3. The greatest mineralization occurred in the purely aerobic version; 32.1% of the initial organic matter was mineralized. A little less, 31.9%, was mineralized in the version with 20 days of preliminary anaerobic incubation and 39.7%, with 10 days of preliminary anaerobic incubation. The least mineralization of the substrate at all was under anaerobic conditions, 26.4%. Less humic acid and fulvic acid was found in this version than in the initial raw material. The  $C:N_{org}$  ratio is large in this version.

With aerobic incubation during the entire test or at the beginning, new humic acids formed, the  $C:N_{org}$  ratio decreased sharply and the indicated stability of the organic matter increased. All these data show that the maximum humification took place in the version with 10 days of preliminary incubation. The 20-day preliminary incubation was less profitable than the 10-day and less profitable /116 than aerobic incubation alone.

In study of these ratios, it was found that, with preliminary anaerobic incubation, low molecular weight matter forms, which can be used as the initial material for synthesis of humus matter, and that the total number and number of species of aerobic microbes gradually increases. In this case, of course, the production of the enzymes necessary for humus synthesis decreases. With 10 days of anaerobic incubation, this effect is expressed little. The ratio of the number of aerobic microbes to the number of anaerobic ones after 60 days of aerobic incubation was approximately 17, and this ratio decreased negligibly, to 15, in the 10-day preliminary anaerobic incubation, with subsequent 50-day aerobic incubation. With 20 days

of preliminary anaerobic incubation and 40 days of subsequent aerobic incubation, this ratio was 5 and, with 60 days of anaerobic incubation, it was even less than 1.

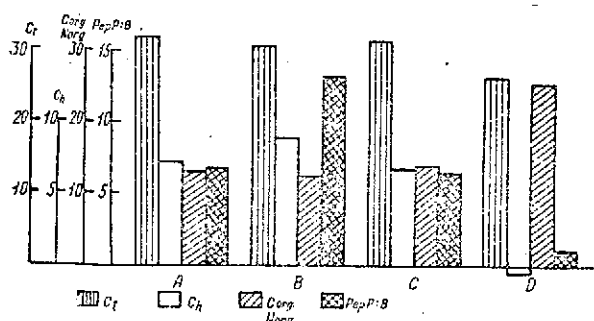


Fig. 3. Characteristics of test versions with preliminary anaerobic incubation and subsequent aerobic incubation: C = mineralization of C;  $C_h$  = C of humic acids formed;  $C:N_{org}$  = the ratio of organic C to organic N;  $Pep:B$  = the ratio of potential and basal respiration; A is 60 days of aerobic incubation; B is 10 days of anaerobic + 50 days of aerobic incubation; C is 20 days anaerobic + 40 days aerobic incubation; D is 60 days of anaerobic incubation

The respiration values differ, upon recalculation to a unit number of microbes. At the end of the test, one million live microbe cells gave off 108  $CO_2$  per hour on the average with continual aeration, 29.9 in the version with 10 days preliminary incubation, 213 in the version with 20 days preliminary incubation and 2970 in the anaerobic version. The respiration values can be evaluated, not only as an expression of the total mineralization of the substrate, but as an expression of the stability of the

organic matter used [Novák, 29-31; Sauerbeck, 51]. Thus, these data/117 indicate a maximum of stabilized organic matter in the version with 10 days of preliminary anaerobic incubation. The organic matter in the completely anaerobic version turned out to be the least stabilized.

Preliminary anaerobic incubation turned out to be a successful measure for increasing the amount of initial matter for humus synthesis. It is likely that this increased both the humification rate and productivity in the aerobic phase. This was confirmed by the work of B. Novak and Cizek.

Preliminary anaerobic incubation reduces the number and activity of aerobic microflora necessary for synthesis of humus. Therefore, we attempted to test, under model conditions, whether or

not the depression of humus synthesis can be decreased or completely eliminated by a new inoculation.



Fig. 4. Potential activity of microflora in aerobic and anaerobic incubation, after prolonged anaerobic incubation

substrates after 63-day anaerobic incubation (first columns), after subsequent 7-day anaerobic incubation (second columns) and with repeated inoculation of the sand cultures with the original soil microflora, at the end of the anaerobic phase of the test and 7 more days of aerobic incubation. The results show that the glycolides are 118 completely unhumified under anaerobic conditions (A). Very little complex organic material is humified. The subsequent aerobic phase of the test (B) causes production of humic acids. This production increases considerably, after inoculation with fresh soil microflora (C).

It can be concluded from the results of these tests that formation of low molecular weight organic matter takes place in the anaerobic phase, which can be gradually incorporated into the high molecular weight humus substances. The aerobic phase allows the required energy for the synthetic process. Synthesis occurs, by means of complex enzymatic reactions. The necessary enzymes are supplied by the microbes, complex microflora, in which aerobic groups of microbes cannot be absent.

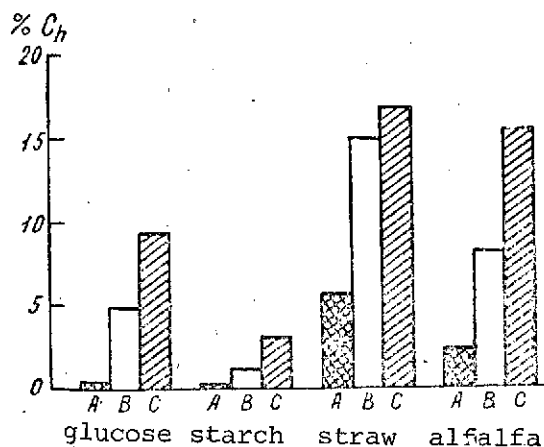


Fig. 5. Formation of humus after 63 days of anaerobiosis (A) and after a further 7 days of aerobiosis, without addition (B) and with addition (C) of fresh soil microflora

Although arguments on the importance of aerobic and anaerobic processes have been taking place on the pages of scientific journals for several decades, nevertheless, the point of view of the energetic formation of humus, which can explain the causes of these arguments and explain the results obtained, have been discussed very little, and they have even been ignored.

Therefore, I consider it to be useful to describe the function of the energy changes, as well as the transfer of energy in humification of the substrate, in greater detail.

It is well known that endothermic reactions can obtain energy directly from the exothermic substrate change reactions, as well as by means of compounds, which are suitable for energy transfer. These compounds, by transferring specific ones of their groups, /119 can transfer a relatively large amount of energy; therefore, they usually are called high-energy compounds; the bonds forming compounds between the group transferred and the residue of the molecule usually are called high energy bonds. These names are not particularly appropriate from the point of view of physical chemistry, but they usually are used everywhere; therefore, we will use them subsequently.

Obtaining energy for endothermic reactions from the conjugate exothermic reactions is limited to systems, in which the oxidation-reduction potentials of both reacting systems are close together; a system supplying energy must have a value of  $E$ , which is more negative than that of the system receiving the energy.

The energy transferred by means of high-energy bonds does not depend on the oxidation-reduction potential. Therefore, synthesis of these high-energy bonds is of special importance, both in biology generally and in synthesis of humus. The most significant of these bonds are the high-energy phosphate bonds, especially those in ATP.

In anaerobic processes, there will be very much less synthesis of high-energy bonds than in aerobic processes. For example, 4 ATP are produced by 1 hexose molecule in glycolysis, of which 2 molecules are consumed in the preceding phosphorylation, so that the pure energy gain is only 2 ATP. In aerobic processes, there are no stoichiometric relations between ATP synthesis and glycolysis, but 36-38 ATP usually are produced by 1 hexose molecule in an aerobic process. The difference between the aerobic and anaerobic processes, from the point of view of energy production, is so great that all the previous test results can be easily explained.

For the purpose of a precise test of our conclusions, we considered it necessary to compare, not only aerobic and anaerobic processes in humification, but also to compare these processes with versions, in which the cytochrome system is inhibited under aerobic conditions and, in this manner, the terminal oxidation and production of energy by the oxidative phosphorylation system, which is a rich source of high-energy bonds under aerobic conditions, is stopped.

We conducted tests, in which ground straw, suspended in a mineral nutrient solution, inoculated with soil microflora, was used as the substrate. Versions A and B were kept under aerobic conditions and C and D, under anaerobic conditions. KCN was added to versions B and D, as a terminal oxidation inhibitor.

The results of the test are presented in Figs. 6 and 7. Mineralization of the substrate, immobilization of N, the humification rate and productivity, stability of organic matter indicated

by respirometric test and the degree of humic acid molecule condensation, indicated by the light coefficient, were significantly higher in the aerobic, noninhibited version than in the anaerobic version. Both inhibited versions hardly differed from the anaerobic, non-inhibited version.

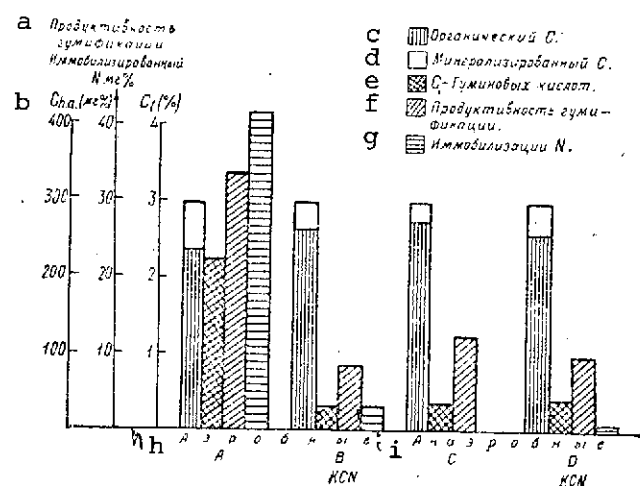


Fig. 6. Characteristics of test versions with terminal oxidation inhibition by potassium cyanide (KCN). Versions: A. aerobic noninhibited; B. aerobic inhibited; C. anaerobic noninhibited; D. anaerobic inhibited.

Key: a humification productivity and immobilized N, mg %  
 b C<sub>ha</sub> (mg %)  
 c organic C  
 d mineralized C  
 e humic acid C  
 f humification productivity  
 g N mobilization  
 h aerobic  
 i anaerobic

Thus, inhibition of terminal oxidations completely eliminates the advantage of aerobic incubation and decreases the rate of biochemical change of the substrate and, with it, humification to the level of anaerobic processes.

It might be proposed that potassium cyanide also inhibits the activity of extracellular oxidases (polyphenol oxidase, peroxidase, laccases, catalases, etc.), which some authors (Scheffer, 52, 53; Flaig, 4; Haider, Lim, 7; Haider, Grabe, 8 and others) consider to be "humifying enzymes."

Therefore, we conducted a similar series of tests, using sodium azide NaN<sub>3</sub>, which, in distinction from KCN

inhibits only oxidative phosphorylation, without disruption of the terminal oxidation processes or activity of the extracellular oxidases, as well as of other enzymes with iron or copper warfarin bonds.

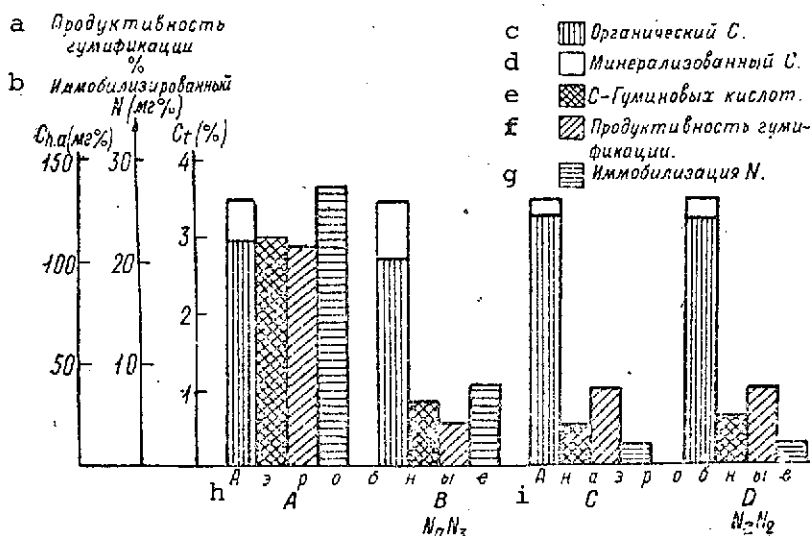


Fig. 7. Organic matter stability (Ng:B) indicated by light coefficient of humic acids ( $Q_{4:6}$ ) of tests with  $\text{NaNO}_3$  incubation [Key same as in Fig. 6]

In distinction from the preceding test, the rate of mineralization was greater in the aerobic, inhibited version than in the aerobic, noninhibited one. The amount of humic acids formed here was considerably less than in the aerobic, noninhibited version, and only a little greater than in both anaerobic versions. The humification productivity, as a consequence of high mineralization of the substrate, was least of all in the aerobic, inhibited

version. The relatively great stability of organic matter in this version is apparently not a result of humus synthesis, but a result of considerable mineralization of the easily used parts of the straw. This confirms the light coefficient of the extracted humic acids, which indicates a low degree of condensation of the molecules. It approaches the values in the anaerobic versions more closely than those in the anaerobic, noninhibited version.

The results of these tests show that the aerobic phase of humification is tremendously more important, as a process supplying the energy necessary for humus matter synthesis, than as an oxidation process.

#### Functions of N and P in Humification

From a test, carried out in our Institute, the effect of nitrogen (N) and phosphorus (P) on humification, with addition of starch to the soil, can be demonstrated. This test had the following variations:

- A - control soil
- B - soil with 1% starch added
- C - " " 1% " , 50 mg %  $P_2O_5$  (i.e., 14.5 mg % P) added
- D - soil with 1% starch, 50 mg % N added
- E - " " 1% " 14 mg % P and 50 mg % N added.

All the variations were kept under aerobic conditions, for 21 /122 days at a temperature of 28°.

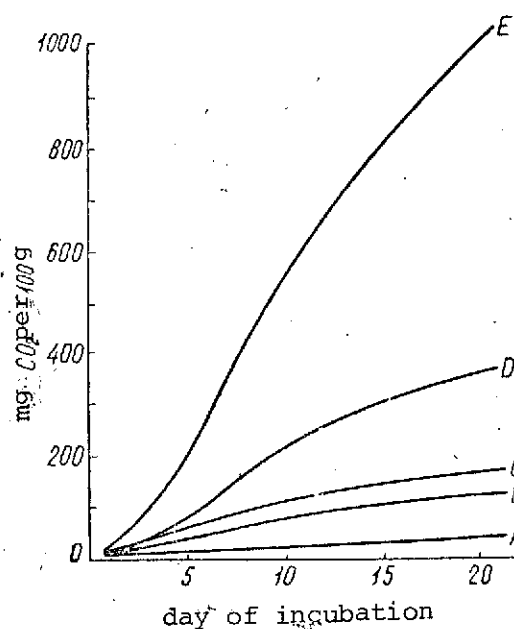


Fig. 8. Course of mineralization in test with addition of starch, N and P to the soil

- A - soil without additive, control
- B - " + 1% starch
- C - " + 1% " + 14.5 mg % P
- D - " + 1% " + 50 mg % N
- E - " + 1% " + 14.5 mg % P + 50 mg % N

The course of mineralization of the organic matter during incubation is presented in Fig. 8. Addition of starch alone increases the total mineralization by almost three times that in the control version, by the end of the period. Addition of P accelerates mineralization, but not as productively as does addition of N. The combined action of N and P on mineralization of starch was three times greater than the sum of the effects of N and P introduced separately.

The addition of phosphate increased the capacity of the soil microflora to synthesize new organic matter. Addition of a

glycide alone increased the amount of fulvic acids, but it decreased the amount of humic acids and also decreased the extent of polymeri-

zation of the humic acids. By addition of N and P, the amount of humic acids and fulvic acids practically equaled those of the control version, but the extent of polymerization of the humic acids/123 is less here than in the control. Only with joint application of C, N and P did we obtain a greater amount of humic acids and fulvic acids than in the control. In this case, the degree of polymerization of the humic acids, even in this variation, does not reach the degree of polymerization of these acids in the control.

### Conclusion

The results obtained can be used, both in preparation of various organic fertilizers and for regulation of the change of organic matter in the soil by microorganisms, by means of agricultural engineering and fertilization measures. The results of all of these studies have been used with great success here, for the time being, only in tests and in individual agricultural enterprises.